American Fern Journal 104(1):1–6 (2014)

A Pluviometric Fern Spore, Fungal Spore, and Pollen Trap

FELIPE GÓMEZ-NOGUEZ
Área de Botánica Estructural y Sistemática Vegetal, Departamento de Biología, Universidad Autónoma Metropolitana-Iztapalapa, Avenida San Rafael Atlixco 186, Colonia Vicentina, Delegación Iztapalapa, 09340 México, D. F., México, e-mail: tindalo@xanum.uam.mx

BLANCA PÉREZ-GARCÍA
Área de Botánica Estructural y Sistemática Vegetal, Departamento de Biología, Universidad Autónoma Metropolitana-Iztapalapa, Avenida San Rafael Atlixco 186, Colonia Vicentina, Delegación Iztapalapa, 09340 México, D. F., México, e-mail: bpg@xanum.uam.mx

ANICETO MENDOZA-RUIZ
Área de Botánica Estructural y Sistemática Vegetal, Departamento de Biología, Universidad Autónoma Metropolitana-Iztapalapa, Avenida San Rafael Atlixco 186, Colonia Vicentina, Delegación Iztapalapa, 09340 México, D. F., México, e-mail: amr@xanum.uam.mx

ALMA OROZCO-SEGOVIA
Departamento de Ecología Funcional, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Circuito Exterior, Ciudad Universitaria, México, D. F., México, e-mail: aorozco@ecologia.unam.mx

ABSTRACT.—Although rain is the most important agent in airborne biological particle deposition most of the current sampling traps retain palynomorphs (fern spore, fungal spore, pollen, among others) but are unable to measure precipitation. The objectives of this study are to present a new simple pluviometric/gravimetric spore/pollen trap and propose a spore/pollen-density rain method, based on the particle frequency and sampling area, which would facilitate ecological inferences about rainfall and Biological Airborne Particle (BAP) deposition, so as to avoid the overrepresentation of the percentage and diverse aerobiological methods. Relative to other spore traps, our proposed trap is simple to build, easy to mount in the field, easy to carry, maintenance free, and requires no energy source. In addition, our trap records rainfall volume, and the quantity of spores captured can be expressed in terms of area-density (particle m^{-2}). The rainfall measured with the trap had no significant differences with the precipitation volume obtained from the pluviometer of the Automatic Meteorological Station at Zacualtipán, Hidalgo, Mexico.

KEY WORDS.—Airborne spore, biological airborne particle, biological particle deposition, efficient spore trap

Aerobiology is a recent scientific discipline that studies the diversity and concentration of biological particles (e.g., pollen, fungal spores, and fern spores) that are transported passively by the atmosphere (Latorre and Caccavari, 2010). Most aerobiological studies focus on the variation of pollen and fungal spore concentrations because of the allergenic effect of these particles (i.e., pollinosis) on humans, and in order to assess the potential for the spread of fungal disease on economic crops (De Benito and Soto, 2001; Lacey and West, 2006). Research has resulted in the development of specialized palynomorph traps, such as the Tauber trap (Tauber, 1974), which is used to analyze pollen deposition (Levetin *et al.*, 2000), and the Burkard volumetric trap (Hirst, 1952), which was designed to study airborne pollen. Both of these devices are commonly used in many types of research (e.g., Caulton *et al.*, 2000; Hicks, 1999; Kasprzyk, 2004; Yang and Chen, 1998), as are specialized traps, such as the rotorod trap (Murray *et al.*, 2007), the cyclone spore trap (Tate *et al.*, 1980), and others reviewed by Gregory (1961) and Lacey and West (2006).

Although these types of traps can be programmed to survey the air for different periods of time, they are expensive and require energy sources unavailable in some tropical environments (Gupta and Chanda, 1991; Potter and Rowley, 1960). Furthermore, they can be lost or stolen during prolonged field surveys. For these reasons, these traps are mostly used in urban areas, on the rooftops of available buildings (Estrella *et al.*, 2006; Latorre and Caccavari, 2010; Ong *et al.*, 2011; Ting *et al.*, 2010). The use of simpler traps has been reported, such as moss clusters on trees (Limón, 1980), soil samplers (Anupama *et al.*, 2002; Tovar-González, 1987), and exposed petri dishes containing different nutritional media (Brown, 1971). However, simpler traps cannot be used to determine the palynomorph influx, and have low local flora representation (Tejero-Díez *et al.*, 1988). Other traps, such as adhesive slices, have low uptake efficiency in prolonged surveys because of sampling area saturation and particle loss due to rain-washing (Melhem and Makino, 1978). Bush (1992) proposed an inexpensive, phenologically accurate gravimetric palynomorph trap composed of a funnel (sampling area) and a carafe; this trap was later improved by Gosling *et al.* (2003), but both of these traps have the disadvantage of not being able to record pluviometric values. Rainfall is the most important meteorological factor in particle deposition (Ramírez-Trejo, 2002; Ramírez-Trejo *et al.*, 2004; Simabukuro *et al.*, 1998, 2000), yet samplers are unable to measure rainfall.

To preserve the pluviometric values associated with a spore-rain survey and to obtain more ecological inferences about rainfall and palynomorph depositions, we modified and simplified the Bush-Gosling trap.

Our trap consisted of a funnel 9 cm in diameter attached to a two-liter carafe (Fig. 1 A and B). To prevent the evaporation of the rainfall deposited in the trap, a segment of PVC tube 15.5 cm (6.5 in) in diameter was added, surrounding the trap and serving as a base. A mosquito net or another type of mesh could be used to cover the funnel and thus prevent major detritus buildup. Moreover, the cylinder–base top edge should be beveled to avoid over representation of rainfall by splashing.

The uptake area (top of the funnel) can be calculated as $A=\pi r^2$; 63.61725124 cm^2 thus, the total rainfall can be expressed in millimeters by the following equation: mm = V/A, where V is the final volume of rain collected expressed in mm^3 and A is the uptake area expressed in mm^2. In addition, the particles may be expressed in density (particles/sampling area), which facilitates analysis and avoids the problems related in the percentage method used by Simabukuro *et al.* (2000).

The advantage of this trap is that it allows better aeropalynological interpretations with the best airborne particle deposition factor (rain). To test

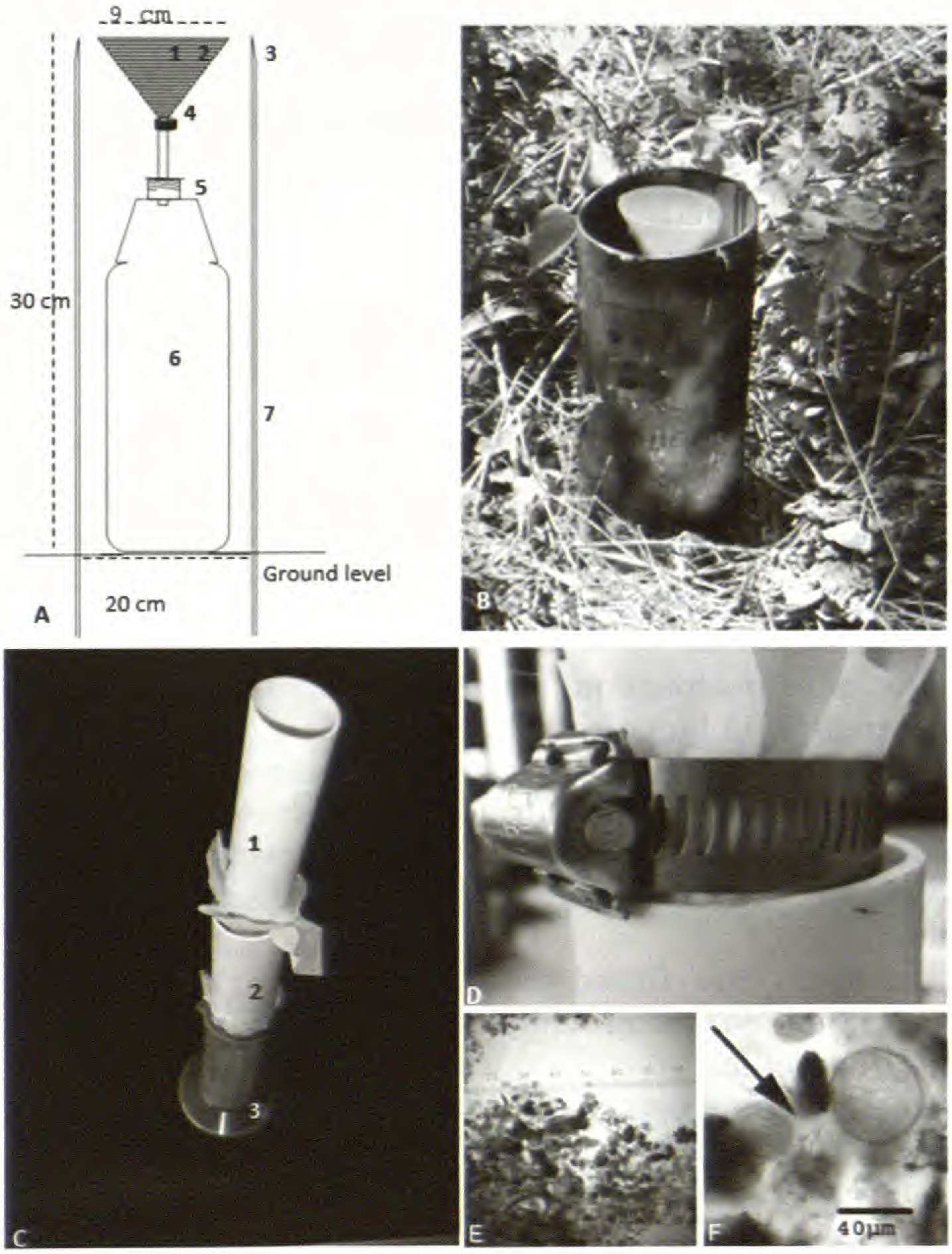

FIG. 1. Pluviometric spore/pollen trap. A. Pluviometric spore/pollen trap diagram: 1) funnel, 2) screen mesh, 3) beveled top edge PVC tube, 4) security seal to secure the mesh, 5) recapping with a hole for the funnel tube, 6) carafe with a 2 L capacity, 7) wall of the PVC tube. B. Trap mounted at an experiment site. C. Measuring and filtering of the trap content with different sized mesh: 1) 100 µm mesh, 2) 20 µm mesh, 3) graduated cylinder. D. Details of simple filter build up by a PVC tube segment, with a 100 µm mesh attached with a clamp. E. Particles captures with the device at 10 × magnification. F. Striate trilete fern spore aff. *Alsophila firma* (Baker) D. S. Conant (arrow), surrounded by diverse pollen grains at 100 × magnification.

durability and efficiency of the trap, we used it to determine the spore rain in an area near the Malila River in the state of Hidalgo, Mexico. The trap was left in the field in its cylinder-base with monthly trap changes, which did not result in damage from environmental conditions.

To vary the sampling period, one can change the carafe capacity and funnel size according to the total rainfall observed in previous years. Because we

TABLE 1. Total number of fern spores captured by six traps, mean precipitation, and spore density in a monthly one-year survey (March 2009 to February 2010). PST =Pluviometric Spore Trap.

Period	Total fern spore counted	PSTM mean precipitation (mm)	Fern spore density (spore m^{-2})
March 2009	621	10.58	16269.2
April 2009	335	10.27	8776.4
May 2009	228	73.09	5973.2
June 2009	274	115.66	7178.3
July 2009	123	7.86	3222.4
August 2009	224	226.33	5868.4
September 2009	129	204.61	3379.5
October 2009	83	154.31	2174.4
November 2009	66	55.80	1729.1
December 2009	101	47.42	2646.0
January 2010	149	98.50	3903.5
February 2010	129	24.49	3379.5

knew the maximum precipitation rates in our study site, we selected a volume capacity of two liters in order to avoid overflow.

With this trap, the palynomorphs and rain remained in the carafe and were later separated by filtration with different size mesh in the laboratory (Fig. 1 C and D). One hundred μm mesh was used to remove medium detritus and 20 μm mesh was used to collect the particles of interest. In addition, rainfall volume was measured.

The 20 μm mesh was rinsed with 70% ethanol, and the ethanol was collected in a 15 ml vial. The liquid was then centrifuged at 1500 rpm for 3 minutes. The resulting precipitate was then suspended with 5 ml of 70% ethanol from which semi-permanent preparations were made.

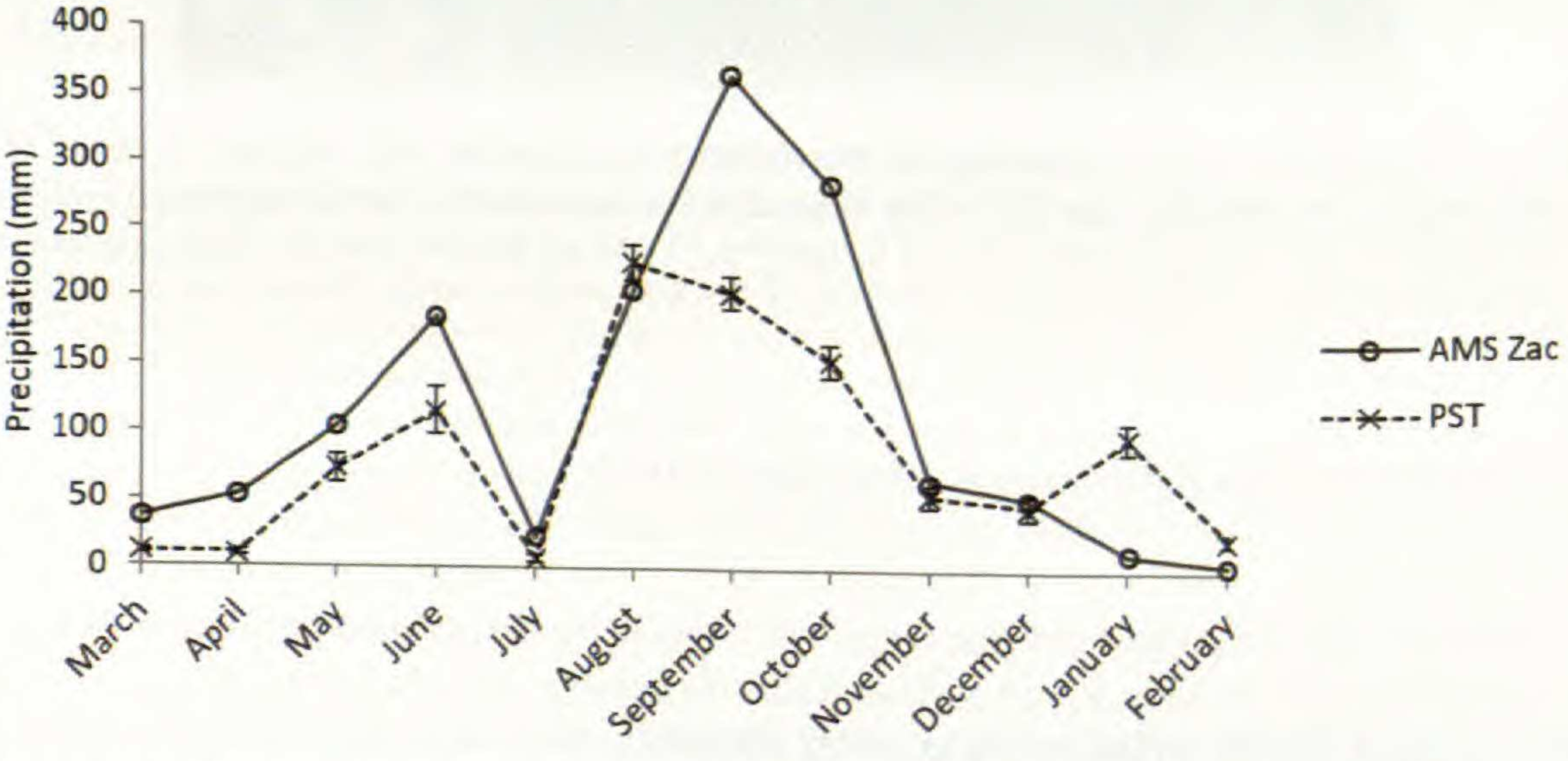

FIG. 2. Monthly precipitation values (mm) of the Automatic Meteorological station at Zacualtipán, Hidalgo, México (AMS Zac) and the mean values of six pluviometric spore/pollen traps (PST) localized at 14 km of AMS Zac, from March 2009 to February 2010.

After a one-year test, the trap showed the ability to capture diverse biological particles such as pollen, fungal spores, and fern spores (Fig. 1 E, F), the latter of which are shown in Table 1. Also the comparison between the precipitation values obtained with six trap devices and those obtained by the pluviometer of the nearest weather station, using a Mann-Whitney U-test, showed no significant differences ($W=52$, $P=0.51$, $\alpha=0.05$, Fig. 2).

Spore perine characters offer more accuracy in the identification of fern taxa and therefore we recommend using a fresh mount rather than the common Erdtman (1960) acetolysis method, in order to preserve the fragile perine of some taxa (Devi, 1980).

This simple trap could help with the determination of phenological processes in tropical environments and thus address future challenges, including understanding the possible consequences of global warming on ferns and their spore dispersal (Mehltreter, 2008).

ACKNOWLEDGMENTS

This paper is part of the thesis project of the first author, who is enrolled in the Biological Sciences Master Program at the Universidad Autónoma Metropolitana. We appreciate the support offered by the university for this study, especially from Alejandro Zavala, Ph. D. We also thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the scholarship provided (224680), the National Meteorological Service and the valuable comments and suggestions of the Victor Steinmann, Ph. D., who helped with revision of the manuscript as well as two anonymous reviewers for their contribution.

LITERATURE CITED

ANUPAMA, K., S. ARAVAJY and S. PRASAD. 2002. Pollen and ecological studies in the Southern Eastern Ghats: a new methodological approach. http://www.ifpindia.org/IMG/pdf/anupama_papers/Anupama_et_al_2002.pdf (accessed 12 Sep 2008).

BROWN, R. M. 1971. Studies on Hawaiian fresh-water and soil algae I. the atmospheric dispersal of algae and fern spores across the island of Oahu, Hawaii, Pp. 175–188 *in* Parker, B. C. and R. M. Brown, eds. *Contributions in Phycology*. University of Virginia, USA.

BUSH, M. B. 1992. A simple yet efficient pollen trap to use in vegetational studies. J. Veg. Sci. 3:275–276.

CAULTON, E., S. KEDDIE, R. CARMICHAEL and J. SALES. 2000. A ten year study of the incidence of spores of bracken, (*Pteridium aquilinum* (L.) Kuhn.) in an urban rooftop airstream in south east Scotland. Aerobiologia 16:29–33.

DE BENITO, R. V. and T. R. SOTO. 2001. Polinosis y aerobiología del polen en la atmósfera de Santander. Alergol. Inmunol. Clin. 16:84–90.

DEVI, S. 1980. The concept of perispore—an assessment. Grana 19:159–172.

ERDTMAN, G. 1960. The acetolisis method, a revised descriptions. Svensk Bot. Tidskr. 54:561–564.

ESTRELLA, N., A. MENZEL, U. KRÄMER and H. BEHRENDT. 2006. Integration of flowering dates in phenology and pollen counts in aerobiology: analysis of their spatial and temporal coherence in Germany (1992–1999). Int. J. Biometeorol. 51:49–59.

GREGORY, P. H. 1961. The Microbiology of the atmosphere. (2nd ed.) Leonard Hill Books Limited and Interscience Publishers Inc., London.

GOSLING, W. D., F. E. MAYLE, T. J. KILLEEN, M. SILES, L. SÁNCHEZ and S. BOREHAM. 2003. A simple and effective methodology for sampling modern pollen rain in tropical environments. Holocene 13:613–618.

GUPTA, S. and S. CHANDA. 1991. Aerobiology of subtropical Eastern Himalayas (Kuseong), India. Aerobiologia 7:118–128.

HICKS, S. 1999. The relationship between climate and annual pollen deposition at northern treelines. Chemosphere Global Change Sci. 1:403–416.

HIRST, J. M. 1952. An automatic volumetric spore trap. Ann. Appl. Biol. 39:257–265.

KASPRZYK, I. 2004. Airborne pollen of entomophilous plants and spores of pteridophytes in Rzeszów and its environs (SE Poland). Aerobiologia 20:217–222.

LACEY, M. E. and J. S. WEST. 2006. The air spora, a manual for catching and identifying airborne biological particles. Springer, Dordrecht, Netherland.

LATORRE, F. and M. A. CACCAVARI. 2010. Pollen diversity in the air of Diamante (Entre Ríos, Argentina). Scientia Interfluvius 1:7–17.

LEVETIN, E., C. A. ROGERS and S. A. HALL. 2000. Comparison of pollen sampling with a Burkard spore trap and a Tauber trap in a warm temperate climate. Grana 39:294–302.

LIMÓN, B. A. 1980. Vegetación y lluvia de polen en el cerro Tetepetl, Estado de México. Tesis de Licenciatura., Facultad de Ciencias Universidad Nacional Autónoma de México, México.

MELHEM, A. and H. MAKINO. 1978. Precipitação polínica na ciudad de São Paulo (Brasil). Hoehnea 7:1–10.

MEHLTRETER, K. 2008. Phenology and habitat specificity of tropical ferns. Pp. 201–221 *in* T. A. Ranker and C. H. Haufler (eds.), *Biology and Evolution of Ferns and Lycophytes*. Cambridge University Press, New York.

MURRAY, M. G., R. L. SCOFFIELD, C. GALAN and C. B. VILLAMIL. 2007. Airborne pollen sampling in a wildlife reserve in the south of Buenos Aires province, Argentina. Aerobiologia 23:107–117.

ONG, T. C., S. H. LIM, X. CHEN, S. D. MOHD DALI, H. T. W. TAN, B. W. LEE and T. CHEW. Fern spore and pollen airspora profile of Singapore. Aerobiologia (2012), http://link.springer.com/content/pdf/10.1007%2Fs10453-011-9217-z.pdf. (accessed 10 Sep 2010)

POTTER, L. D. and J. ROWLEY. 1960. Pollen rain and vegetation, San Agustin plains, New Mexico. Bot. Gaz. 122:1–25.

RAMÍREZ-TREJO, M. R. 2002. Los Bancos de esporas de helechos en diferentes suelos y tipos de vegetación del estado de Hidalgo. Tesis de Maestría, Facultad de Ciencias Universidad Nacional Autónoma de México, México.

RAMÍREZ-TREJO, M. R., B. PÉREZ-GARCÍA and A. OROZCO-SEGOVIA. 2004. Analysis of fern spore banks from the soil of three vegetation types in the central region of Mexico. Amer. J. Bot. 91:682–688.

SIMABUKURO, E. A., L. M. ESTEVES and G. M. FELIPPE. 1998. Fern spore rain at Itirapina (Sp, Brazil): preliminary results. Ínsula (Florianópolis) 27:39–57.

SIMABUKURO, E. A., L. M. ESTEVES and G. M. FELIPPE. 2000. Fern spore rain collected at two different heights at Moji Guacu (Sao Paulo, Brazil). Fern Gaz. 16:147–166.

TATE, K. G., J. M. OGAWA, W. E. YATES and G. STUGERON. 1980. Performance of a cyclone spore trap. Phytopathology 70:285–290.

TAUBER, H. 1974. A static non-overload pollen collector. New Phytol. 73:359–369.

TEJERO-DÍEZ, D., M. REYES-SALAS and E. MARTÍNEZ-HERNÁNDEZ. 1988. Lluvia de polen moderno en un gradiente altitudinal con vegetación templada en el Municipio de Ocuilán, Edo. Mex. (Mex.). Palynol. Paleobot. 1:61–80.

TING, D. Y. P., F. HOPF, S. G. HABERLE and D. M. J. S. BOWMAN. 2010. Seasonal pollen distribution in the atmospheric of Hobart, Tasmania: preliminary observations and congruence with flowering phenology. Austral. J. Bot. 58:440–452.

TOVAR-GONZÁLEZ, R. C. 1987. Lluvia de polen en el volcán Popocatépetl: un estudio a lo largo de un transecto altitudinal. Tesis de licenciatura., Facultad de Ciencias Universidad Nacional Autónoma de México, México.

YANG, Y-L. and S-H. CHEN. 1998. An investigation of airborne pollen in Taipei city, Taiwan, 1993–1994. J. Pl. Res. 111:501–508.